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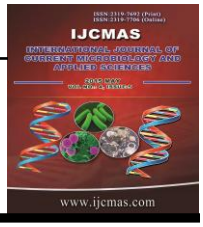

Original Research Article

Assay of Antimicrobial Activity of Leaf Extract of Madhunasini (*Gymnema sylvestre*)

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Keywords

Madhunasini, Antimicrobial activity, Leaves, Pathogenic microorganisms

A B S T R A C T

The aim of this study was to evaluate antimicrobial activity of aqueous leaf extract of *Gymnema sylvestre* against pathogenic microorganisms. Leaves of *G. sylvestre* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with water. The extracts were diluted in dimethyl sulfoxide (DMSO) before testing the antimicrobial activity. The antimicrobial activity of extract for *B. subtilis, S. aureus,* and *A. niger* was determined by agar well diffusion technique. The results of the present study revealed that aq. leaf extract of *G. Sylvestre* exhibited zones of inhibition at 100 mg/ml concentration. Aq. leaf extract of *G. sylvestre* exhibited zone of inhibition zone bacterial species like *B. subtilis* (9.50 mm), *S. aureus* (9.50 mm), and against fungal species like *A. niger* (11.00 mm). In conclusion, study results demonstrated that the aqueous extract of *G. sylvestre* possesses considerable antimicrobial activities against *B. subtilis, S. aureus, and A. niger*. Hence, further experiments like isolation and purification of phytoactives of *G. sylvestre* responsible for antimicrobial activities could be recommended to carried out to further enhance the antimicrobial activities of phytoactives present in aq. leaf extract of *G. sylvestre*.

Introduction

According to World Health Organization (WHO) therapeutic plants would be the best source to acquire an assortment of medications.[1] The data on therapeutic plants from Ayurveda, Unani, Homeopathy and Siddha gives thought that the medicinal plants contain a wide range of ingredients. The medicinal value of plants is because of ingredients like alkaloids, flavonoids, tannins and phenolics.[2] They can be used to treat chronic as well as infectious diseases. Medicinal plants are the main source of pharmaceuticals and healthcare products.[3] Medicinal plants products are used as home remedies to treat specific conditions as well as complex preparations to treat life threatening diseases.[4] The antimicrobial properties of medicinal plants have been examined by some of scientists around the world. Recent exploration survey uncovered

that, medicinal plants are evaluated for biological activities for discovering likely new compounds for remedial use.[5-7] The utilization of plant extracts and phytochemicals, both with known antimicrobial properties, can be of incredible importance in therapeutic medicines.[8] Thus, more investigations relating to the utilization of plants as therapeutic agents thought to be underscored. *G. sylvestre* (Asclepiadaceace), a vulnerable species is a slow growing, perennial, medicinal woody climber found in central and peninsular India. The plant is considered to be a good source of a large number of bioactive substances. *G. sylvestre* leaves contains large number of phytochemicals like terpenoids, saponins, gymnemic acids, gymnemasaponins. The essential oil obtained from *G. sylvestre* leaves exhibited antioxidant and antimicrobial activity.[9] There is a growing demand for *G. sylvestre* leaves in pharmaceutical trade. The active compound gymnemic acid was extracted from leaves and used widely as an antidiabetic, anti-sweetener and anti-hypercholesterolemia. It also has stomachic, diuretic and cough suppressant properties.[10, 11] Thus, literature study evidenced the traditional usage of *G. sylvestre* in pharmaceutical industry. However, very limited work has been done on the antimicrobial activity of this medicinal plant. Hence, the present study was designed with the main aim to evaluate antimicrobial activity of aqueous leaf extract of *G. sylvestre* against pathogenic microorganisms.

Materials and Methods

Plant Material

The leaves of *G. sylvestre* were collected from natural habitat of Karnataka state India.

Preparation of Plant Extract

Leaves of *G. sylvestre* was washed thoroughly under running tap water, dried on paper. Dried leaves were coarsely powdered and subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with water. Extract was concentrated by distilling the solvent in a rotary flash evaporator. The extract was preserved in airtight containers and stored at 4-5^{o}C until further use. The extract was diluted in dimethyl sulfoxide (DMSO) before testing for the antibacterial activity.[12]

Procurement of Cultures

For Antimicrobial activity studies following microbial cultural were used: *Bacillus subtilis* (ATCC 2239), *Staphylococcus aureus* (ATCC 2178), *Aspergillus niger* (ATCC 504). The microbial cultures were procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune.

Antimicrobial Activity

Antimicrobial activity was carried out by agar well diffusion method.[13] Pure cultures of *Bacillus subtilis*, *Staphylococcus aureus*, and the fungal cultures of *Aspergillus niger* were obtained National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune. The mother cultures of *Bacillus subtilis*, *Staphylococcus aureus*, and *Aspergillus niger* were allowed to stand for 24 h in order to reach the stationary phase of growth before the assays. Petri dishes containing the mother cultures with proper sterile Muller-Hinton agar medium was used for bacteria. The media were inoculated to obtain the microorganism concentration of 130 x 10^7 cfu/ml. A sterile filter paper disc was loaded with 40 ml sample (50 mg/ml). The disc was

placed near the edge of the agar surface of the inoculated plate. All the plates were kept at 50 °C for half an hour for diffusion. The plates were then incubated for 24 h at 37 °C and the diameters of growth inhibition zones were measured using distilled water as a blank. Each assay was performed in triplicates on three independent experimental runs. The minimum inhibitory concentration (MIC) of extracts indicating clear inhibition was determined by agar diffusion method.[14] Chloramphenicol (10 µg/ml) was used as standard for the antimicrobial activity.

Results and Discussion

As compared with synthetic drugs, naturally derived drugs are therapeutically active and commercially available.[15] The antimicrobial activity and MIC ranges between 10.00 mg/ml to 100 mg/ml exhibited by aq. leaf extract of *G. sylvestre* extract was as represented in Table 1 and Table 2 respectively. In the antimicrobial studies aq. leaf extract of *G. Sylvestre* exhibited zones of inhibition at 100 mg/ml concentration. Aq. leaf extract of *G.sylvestre* exhibited zone of inhibition zone bacterial species like *Bacillus subtilis* (9.50 mm), *S. aureus* (9.50 mm), and against fungal species like *A. niger* (11.00 mm).

These findings delineated that aq. leaf extract of *G. sylvestre* was active against tested organisms. Hence, the present results of the present research investigation offer a scientific basis for traditional use of aq. leaf extract of *G. sylvestre*. Plants are sources of very potent and powerful drugs with antibacterial properties.[16, 17] Antibacterial assay of Zulu medicinal plants showed that methanolic extracts of *Chelianthes viridis*, *Dioscorea dregeanam*, *Dioscoria silvatica* and *Molianthus cosnlosus* exhibited activity against both Gram positive and Gram-negative bacteria.[18] Samy and Ignacimuthu screened 30 Indian folk medicinal plants used by traditional healers using disc diffusion method.

Table.1 Antimicrobial activity of aq. leaf extract of *G. sylvestre* against various microbial strains

Microorganisms		**Aq. leaf extract of *G. sylvestre***
Gram Positive Bacteria	*Bacillus subtilis*	9.50
Gram Negative Bacteria	*Staphylococcus aureus*	9.50
Fungal Species	*Aspergillus niger*	11.00

Table.2 MIC of Aq. leaf extract of *G. sylvestre* against various microbial strains

Microorganisms		**Aq. leaf extract of *G. sylvestre***
Gram Positive Bacteria	*Bacillus subtilis*	12.50
Gram Negative Bacteria	*Staphylococcus aureus*	12.50
Fungal Species	*Aspergillus niger*	11.50

Among them, the leaf extracts of *Cassia occidentalis* and *Cassia comiculata* exhibited significant broad-spectrum antibacterial activity against *Bacilus subtilis* and *Staphylococcus aureus*.[19] Our findings are comparable to literature findings reported by various research investigators. In the present study, antimicrobial activity of aq. leaf extract of *G. sylvestre* was evident due to clear zone of inhibition against test organisms like *B. subtilis, S. aureus, E. coli, K. aerogenes, A. niger*. The antimicrobial activities of aq. leaf extract of *G. sylvestre* could be mainly accredited to phytoactives of phytochemicals present in different proportions in *G. sylvestre*.

The study results demonstrated thatthe aqueous extract of *G. sylvestre* possesses considerable antimicrobial activities against *B. subtilis, S. aureus,* and *A. niger*. Hence, further experiments like isolation and purification of phytoactives of *G. sylvestre* responsible for antimicrobial activities could be recommended to carried out to further enhance the antimicrobial activities of phytoactives present in aq. leaf extract of *G. sylvestre*.

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